

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C08B 37/08, C08L 5/08, A61K 31/725	A1	(11) International Publication Number: WO 97/04012 (43) International Publication Date: 6 February 1997 (06.02.97)
(21) International Application Number: PCT/SE96/00684 (22) International Filing Date: 28 May 1996 (28.05.96) (30) Priority Data: 08/503,323 17 July 1995 (17.07.95) US (71)(72) Applicant and Inventor: ÅGERUP, Bengt [SE/SE]; Hävelvägen 6, S-756 47 Uppsala (SE). (74) Agent: AWAPATENT AB; P.O. Box 45086, S-104 30 Stockholm (SE).		(81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: POLYSACCHARIDE GEL COMPOSITION (57) Abstract The present invention provides a process for preparing a cross-linked biocompatible polysaccharide gel composition, which comprises: forming an aqueous solution of a water soluble, cross-linkable polysaccharide; initiating a cross-linking of said polysaccharide in the presence of a polyfunctional cross-linking agent therefor; sterically hindering the cross-linking reaction from being terminated before gelation occurs, an activated polysaccharide being obtained; and reintroducing sterically unhindered conditions for said activated polysaccharide so as to continue the cross-linking thereof to a viscoelastic gel. The invention also provides a gel composition obtainable by such a process as well as gel compositions for different medical uses.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

POLYSACCHARIDE GEL COMPOSITIONTECHNICAL FIELD

The present invention relates to the field of bio-compatible polysaccharide gel compositions, and more specifically to a novel process for cross-linking such compositions, a new gel structure thereby being obtained. The new structure imparts improved properties to the previously known gel compositions as well as enables new uses of said compositions, both as such and containing active ingredients.

BACKGROUND OF THE INVENTION

Water-binding gels are widely used in the biomedical field. They are generally prepared by chemical cross-linking of polymers to infinite networks. When using bio-compatible polymers generally a low degree of cross-linking has to be utilized to maintain said biocompatibility. However, often a more dense gel is required to have a proper effect of the active ingredients utilized, and in such a case the biocompatibility will often go lost.

Another valuable property of water-binding gels, or hydrogels, is that peptides and larger biologically active substances can be enclosed therein to the formation of a sustained release composition. However, practical problems have been involved in accomplishing a sufficient maintenance time of the active ingredient, since generally the active ingredient is released at the same rate with which it was dissolved or enclosed in the composition referred to. Furthermore, if such a gel were densified in an attempt to maintain the active ingredient for a longer time, it would rapidly swell in an animal tissue where there is a free access of water.

One of the most widely used biocompatible polymers for medical use is hyaluronic acid. As it is present in identical composition in each living organism, it gives a minimum of reactions and allows for advanced medical

uses. As a consequence thereof it has been the subject of many modification attempts. Thus, it has been cross-linked with agents such as aldehydes, epoxides, polyaziridyl compounds and divinylsulfone (Laurent et al, Acta Chem. Scand 18 (1964) No 1, p. 274; EP 0 161 887B1; EP 0 265 116A2; and US 4,716,154).

In WO 87/07898 there is disclosed a reaction of a polysaccharide with a polyfunctional epoxide, removal of excess of said epoxide and finally drying operation to cross-link said polysaccharide into a film, powdered material or similar dry product. However, there is no suggestion therein to dilute the activated polysaccharide and then reconcentrate the same to the desired density or consistency which is then substantially permanent.

US 5,128,326 discloses a number of modified hyaluronic acids for use as depot pharmaceuticals. The disclosed methods of "charging" the gel preparations are all based on a diffusion of the active ingredient into the gel and then a release thereof with the same diffusion constant. Contrary thereto the present invention involves a dissolution of the active ingredient followed by a densification or concentration of the gel composition until no or a very minor diffusion of said active ingredient takes place.

US 5,399,351 discloses mixtures of gel and polymeric solutions, said solutions being utilized to improve the rheological properties of the gel. However, also in this case reversibly compressed gels are disclosed, as can be gathered from e.g. col. 6, lines 53-58.

SUMMARY OF THE INVENTION

According to the present invention it has unexpectedly been found that polysaccharide gel compositions having a novel structure and thereby new, outstanding properties can be obtained by using a new technique for the cross-linking thereof. Said new cross-linking technique enables a versatile control of the structure and properties of the manufactured polysaccharide gel composition,

which in turn makes it possible to tailor the final composition for the intended purposes.

More specifically, one object of the present invention is to provide a process for preparing a cross-linked polysaccharide gel composition, the biocompatibility of which can be retained in spite of a high degree of cross-linking or polymerisation.

Another object of the invention is to provide a polysaccharide gel composition with viscoelastic properties in spite of being cross-linked to a substantial degree.

Yet another object of the invention is to provide a polysaccharide gel composition which is more or less irreversibly densified or concentrated, i.e. which does not swell substantially or only to a limited degree when contacted with water.

Still another object of the invention is to provide a polysaccharide gel composition enclosing a biologically active substance for use as a sustained release composition or a depot composition.

Another object of the invention is to provide polysaccharide gel compositions containing a variety of biologically active substances for uses as medical or prophylactic compositions for different purposes.

Yet another object of the invention is to provide uses of the compositions referred to for the manufacture of medical or prophylactic compositions as well as for administration to mammals, especially humans.

Yet another object of the invention is to provide a partially cross-linked activated polysaccharide gel composition as obtained as an intermediate in the above-mentioned process according to the invention, which intermediate can be finally cross-linked in situ at any desired site.

These and further objects of the invention will become apparent by the more detailed description thereof presented below.

DETAILED DESCRIPTION OF THE INVENTION

According to one aspect of the present invention a process for preparing a cross-linked biocompatible polysaccharide gel composition is thus provided, which process comprises:

- forming an aqueous solution of a water soluble, cross-linkable polysaccharide;
- 10 initiating a cross-linking of said polysaccharide in the presence of a polyfunctional cross-linking agent therefor;
- sterically hindering the cross-linking reaction from being terminating before gelation occurs, an activated polysaccharide thereby being obtained; and
- 15 reintroducing sterically unhindered conditions for said activated polysaccharide so as to continue the cross-linking thereof up to a viscoelastic gel.

In other words the new process according to the present invention involves a cross-linking of a water-soluble, cross-linkable polysaccharide in at least two steps or stages, where the cross-linking reaction is discontinued before the gelation is initiated, said discontinuance being accomplished by sterically hindering said cross-linking reaction. The cross-linking reaction is then continued in a second step by reintroducing sterically unhindered conditions.

- 20 Thus, firstly it has unexpectedly been found that by said sterical hindrance an activated polysaccharide is obtained, the cross-linking or polymerization of which can be continued merely by reintroducing sterically unhindered conditions therefor. Secondly, it has also unexpectedly been found that the polysaccharide gel composition obtained thereby does not form the compact, dense structure which would have been obtained if performing
- 25
- 30
- 35

the corresponding cross-linking reaction in one single step to a fully cross-linked gel but rather a viscoelastic gel. Furthermore, as was mentioned above, the new gel structure obtained by the present invention represents a substantially irreversible gel structure which does not swell to any appreciable extent in contact with water or any other aqueous medium. Generally this means that said reswelling is less than 10% by volume based on the volume as obtained from the process claimed.

Although the invention is not bound by any theory it may be that the new structure obtained by the present invention is a combination of cross-linking between existing polymer chains and an elongation of existing chains rather than a very dense network giving a very rigid structure. What may suggest such a mechanism is the fact that a viscoelastic product is obtainable by the invention.

As used herein the term "sterically hindering the cross-linking reaction" should be interpreted in a broad sense, i.e. it need not necessarily be a complete hindrance but in many cases rather a partial hindrance of the reaction referred to. That is, what is important is that the rate of cross-linking is substantially reduced to enable the final cross-linking reaction to take place with new reaction sites involved.

Similarly, the term "reintroducing sterically unhindered conditions" should also be interpreted broadly, which generally means that said sterically unhindered conditions need not necessarily be exactly the same sterical conditions as were used when initiating the cross-linking reaction. Thus, what is generally of importance is that said sterically unhindered conditions enable more rapid reactions to take place than said sterically hindered conditions.

The sterical hindrance of the cross-linking reaction should be obtainable in different ways, but a preferred embodiment of the invention in this respect is repre-

sented by the case where the sterical hindrance comprises diluting the aqueous medium in which the cross-linking reaction is performed, to accomplish a lower concentration of the polysaccharide in said medium.

5 To reintroduce sterically unhindered conditions should also be possible in different ways, but a preferred embodiment in this respect is the case which comprises evaporating the aqueous medium in which the cross-linking reaction is performed, to accomplish a higher
10 concentration of the polysaccharide in said medium. Another preferred embodiment in this respect is represented by the case comprising dialysing the aqueous medium in which the cross-linking reaction is performed.

According to a preferred embodiment of the invention
15 the sterical hindrance of the cross-linking reaction is accomplished before the cross-linking agent has been consumed. This in turn generally also means that the reintroduction of sterically unhindered conditions is initiated in the presence of said non-consumed cross-linking
20 agent.

The sterical hindrance of the cross-linking reaction can generally be started or performed in the range of 50-90% of the total gelation time used in the process according to the invention, consideration also being taken
25 to suitable elasticity or consistency for the intended use of the composition.

The inventive idea should be applicable to any biocompatible polysaccharide that is cross-linkable and soluble in an aqueous medium. Thus, the term "water soluble"
30 ble" should be interpreted in a broad sense, pure water not necessarily being necessary. That is, aqueous solution means any solution wherein water is the major component. A preferred sub-group of polysaccharides in connection with the invention is, however, a glucose amine glucan, of which hyaluronic acid is a specially interesting
35 example.

The cross-linking agent to be used in connection with the invention is any previously known cross-linking agent useful in connection with polysaccharides, consideration being taken to ensure that the biocompatibility prerequisites are fulfilled. Preferably, however, the cross-linking agent is selected from the group consisting of aldehydes, epoxides, polyaziridyl compounds, glycidyl ethers and dividylsulfones. Of these glycidyl ethers represent an especially preferred group, of which 1,4-butandiol diglycidylether can be referred to as a preferred example. In this connection it should also be mentioned that "polyfunctional" includes difunctional.

The initial cross-linking reaction in the presence of a polyfunctional cross-linking agent can be performed at varying pH values, primarily depending on whether ether or ester reactions should be promoted. Preferably this means that said cross-linking reaction is performed at an alkaline pH, especially above pH 9, e.g. in the range of pH 9-12, when promoting ether formations. When promoting ester formations said cross-linking reaction is preferably performed at an acidic pH, especially at pH 2-6.

One interesting aspect of the invention is represented by the case where the prepared cross-linked polysaccharide gel composition is utilized as such as the invention enables the manufacture of a viscoelastic composition. Such a viscoelastic composition is for instance useful in eye surgery, as a synovial fluid substitute, as eyedrops, etc, and as has been referred to above the present invention makes it possible to tailor the viscoelastic properties for such uses. Thus, by utilizing the sterical technology according to the present invention it is possible to obtain chain extensions, chain branchings, cross-links, etc, in a more controlled way than by the previously used techniques with more or less randomized coupling sites.

Furthermore, through the fact that the gels obtained in accordance with the invention do not retain their original volume in the presence of an aqueous medium, the new products do not cause any interfering or negative
5 volume effects in these or other medical uses.

In accordance with the present invention it is also possible to include within the polysaccharide gel composition any biologically active substance for which a polysaccharide gel carrier is desired or accepted. In
10 this context the dilution-concentration technique used in the process claimed enables the enclosure of said biologically active substance before subjecting the polysaccharide to sterically unhindered conditions. That is, while sterically unhindered conditions generally means a
15 concentrating operation, such an operation means that the biologically active substance will be present in a phase that is more compacted than when said substance was included in said carrier. In other words the biologically active substance can be retained much longer as compared
20 to previously known gel cross-linking reactions. Thereby a better sustained release profile for the active substance is obtainable.

In connection with the incorporation of the biologically active substance into the composition an adjustment
25 of the conditions to physiological pH and salt conditions is preferably performed to have a preparation ready for medical use. Such a physiological adjustment is preferred also as concerns the reaction conditions as the second step of the process has been found to proceed well under
30 suchg conditions.

The invention should not be limited in any respect as to the biologically active substance as compared to the use of said substance in prior cases. In other words the condition to be treated should be decisive for the
35 specific substance to be selected.

However, interesting substances in connection with the invention can be selected from the group consisting

of hormones, cytokines, vaccines, cells and tissue augmenting substances. Thus, the unique combination of properties of the new gel composition according to the present invention makes it extremely advantageous in connection with these substances, i.e. primarily thanks to outstanding depot or sustained release properties and non-swelling properties.

One interesting group of biologically active substances thus is tissue augmenting substances as a polysaccharide gel is an advantageous carrier therefor. Further details concerning such products can be found in WO94/21299. More specifically, a preferred tissue augmenting substance comprises a polymer selected from collagen, starch, dextranomer, polylactide and copolymers thereof, and poly- β -hydroxybutyrate and copolymers thereof.

In connection with hormones erythropoietin and calcitonin are especially preferred.

The process according to the present invention also enables the incorporation of the biologically active substance by chemical reaction with the polysaccharide gel structure, or the cross-linking agent therefor, provided that said active substance contains functional groups reactive therewith. Unique properties or combinations of properties can thereby be obtained as in such a case for instance the release rate of the active ingredient will be decided by the degradation or decomposition of the polymer network rather than by the dissolution or migration rate for the substance referred to from the gel network.

A modification of last-mentioned technique in accordance with the invention means that the functional groups of the active substance may have been prereacted with a cross-linking agent for the polysaccharide. Preferably the same cross-linking agent is used as is used in the cross-linking of the polysaccharide.

Since the process of the present invention provides a new polysaccharide gel composition or structure, another aspect of the invention is represented by the novel polysaccharide gel composition prepared. In this respect
5 the scope of protection encompasses not only the polysaccharide gel composition whenever prepared by said process but also any polysaccharide gel composition which is obtainable by a similar technique.

Expressed in another way the present invention also
10 provides a cross-linked biocompatible polysaccharide gel composition, which is obtainable by cross-linking of a cross-linkable polysaccharide with a polyfunctional cross-linking agent therefor in two steps, the first cross-linking step being terminating before gelation occurs, by a sterical hindrance of the cross-linking reaction,
15 and the second cross-linking step being initiated by reintroducing sterically unhindered conditions for said cross-linking reaction to continue the same up to a viscoelastic gel.

20 All those features which have been presented as preferred or interesting features in connection with the claimed process are applicable also to said polysaccharide gel composition per se and need not be repeated once more.

25 Still another aspect of the invention is represented by the case where an intermediate product is obtained by postponing the final step of the cross-linking reaction with sterically unhindered conditions to a later stage or site, for instance at the ultimate use of the composition.
30 Thus, it has been found that the intermediate product obtained after the sterical hindrance of the cross-linking reaction possesses such a stability that the termination of the cross-linking reaction can be performed at a later stage.

35 The invention also relates to the composition defined above for use as a medical or prophylactic composition.

Another aspect of the invention is the use of said composition for the manufacture of a medical or prophylactic composition for any of the above-mentioned specific medical or therapeutical purposes, tissue augmentation and hormone treatment of a mammal, especially a human being, being preferred applications.

Finally, the invention relates to a method of medical or prophylactic treatment of a mammal, especially a human being, which comprises administering a composition as defined above to a mammal in need of such a treatment.

EXAMPLES

The invention will now be exemplified by the following non-limiting examples.

Example 1

Activation of the polymer.

a. Under alkaline conditions

Polysaccharide in the form of 10 g of hyaluronic acid prepared by fermentation of Streptococcus were dissolved in 100 ml of 1% NaOH pH >9. Cross-linking agent in the form of 1,4-butanediol diglycidylether was added to a concentration of 0,2%. The solution was incubated at 40°C for 4 hours.

b. Under acidic conditions

The experiment was performed as in 1a but at an acidic pH of about 2-6 by the addition of 1% of acetic acid to the solution instead of NaOH according to 1a.

Example 2

Preparation of a viscoelastic gel.

The incubates according to 1a and 1b were diluted to a volume which was twice the volume finally desired or about 0,5-1% and were neutralised. The gel was then rotary evaporated to a viscoelastic gel.

Example 3

Preparation of a gel containing dextranomer particles.

The incubates according to 1a and 1b were diluted to a strength of 1% and 20 g dry dextranomer particles (Sephadex®25, Pharmacia) were mixed with the solution,

the particles being enclosed by the cross-linking of hyaluronic acid polymer in a few minutes as a consequence of the concentration of hyaluronic acid which is accomplished by an absorption of water by the dextranomer
5 beads.

The viscoelastic gels obtained were stable, autoclavable and injectable by means of thin hypodermic needles.

Example 4

10 Preparation of a gel for use as a depot medicine containing erythropoietin (EPO).

The incubate obtained in Example 1a was diluted to a strength of 1% and the pH was adjusted by the addition of a citrate buffer according to the instructions from the
15 manufacturer (Ortho Biotech Inc., Raritan USA) for a good stability in aqueous solution. 5×10^6 IU of EPO were added under stirring. After evaporating the solution to 1/4 of the volume the polymer had been cross-linked to a depot composition and an amount of 20 000 IU of EPO/ml
20 was recovered.

Example 5

Preparation of a gel for use as a depot preparation containing calcitonin.

Calcitonin from salmon 100 IU/ml (Miacalcic® Sandoz) were admixed with 2% of polymer solution manufactured in accordance with Example 1b and the solution was
25 concentrated to 5% (250 IU/ml) by rotary evaporation. A horse with chronic claudication in the right front leg was treated with an injection of 2 ml s.c. per week during two weeks. In the six weeks following thereafter said
30 horse was free from pains. The serum calcium was lowered with 12% only.

Example 6

35 Preparation of a gel containing heparin to be released in a sustained way.

In a diluted activated polymer according to Example 4 heparin was dissolved in an amount of 5% of the poly-

mer. The mixture obtained was equilibrated for 1 hour, whereupon it was evaporated to 1/4 of the volume. A coagulation inhibiting release thereof was noted during 16 days of incubation in physiological saline.

5

Example 7Preparation of a gel with covalently bonded heparin in a sterically controlled position.

Activated polymer according to Example 1 was precipitated in methanol under vigorous stirring. The fine-threaded precipitation obtained was dried during the night. Heparin was activated in accordance with example 1. After said incubation (4 hours at 40°C) the polymer precipitation was mixed with the activated heparin solution. The mixture was incubated during the night and the following day the gel solution was neutralised, particulated and washed from reactant residues.

The gel formed was able to bind growth factor, interleukin basic Fibroblast Growth Factor (bFGF), but did not show any inhibition of the coagulation of whole blood.

20

Example 8Preparation of a gel containing positively charged groups of chitosan.

Incubation of a mixture of 7,5 g of hyaluronic acid polymer and 2,5 g of chitosan (See Cure® Protan) was performed in accordance with example 1. After a dissolution and a neutralisation a copolymerized viscoelastic solution was obtained. Said solution possessed healing promoting properties after having been applied to a sore slow in healing.

30

Example 9Preparation of a gel which has been sterically coupled.

7,5 g of hyaluronic acid were activated in accordance with Example 1a. In the same way 2,5 g of dextran were activated. The hyaluronic acid was precipitated in methanol, the precipitation then being mixed with 500 ml of a diluted activated 0,5% dextran solution. After stir-

ring and adjustment of pH and salt concentration a viscoelastic solution was obtained. 5 ml of said solution was infused in an Achilles tendon sheath which repeatedly showed inflammation in the form of soreness and
5 "creaking". After four weeks said Achilles tendon problems had disappeared.

Example 10

Preparation of a gel for use as a medicinal depot containing GMCSF.

10 The product was prepared in accordance with Example 5 but instead of calcitonin there was added Granulocyte macrophage - colony stimulating factor, GMCSF (Leucomax®) 1 mg/g polymer.

Example 11

15 Preparation of gel containing killed virus type Influenza A2.

The preparation was performed as in Example 4 but instead of EPO 40 960 HAU killed influenza horse virus per 100 ml of diluted active 1% polymer solution were
20 added. After contraction 4x the preparation contained 1 600 HAU per ml. By a vaccination of more than 100 horses in connection with an epidemic influenza the preparation was found to be highly effective as to protection against infection, which protection was maintained for a long
25 time (more than 6 months).

Example 12

Preparation of a fresh gel containing a living cell suspension.

30 A 5 ml fibroblast culture was mixed with 100 ml of a neutralized solution according to example 1a. The mixture was oxygenated and dried to half the volume. A viscoelastic solution containing living cells was obtained.

Example 13

35 Preparation of a dense micronised gel containing small peptides.

To an activated neutralized gel according to Example 1a there was added 5 mg of a peptide having 12 amino ac-

ids. The gel was evaporated during stirring to 10% and was suspended in mineral oil. After addition of methanol the dry gel particles were filtered off and washed clean from oil residues.

5 Example 14

Preparation of a gel containing the dense micronised gel with small peptides according to Example 13.

To a 1% solution of neutralized polymer activated according to Example 1a microspheres from Example 13 were added. The gel was then evaporated to half its volume. A homogenous injectable and stable gel containing finely dispersed microspheres was formed.

10 Example 15

Preparation of a gel containing spherical polymethylmethacrylate (PMMA) beads having a size of 40 - 120 μ m.

To 5 g of a polymer diluted to 1% and neutralized and activated according to Example 1a 100 mg of spheres of polymethylmethacrylate (PMMA) were added. Evaporation to 3% polymeric gel gave a stable injectable viscoelastic gel.

20 Example 16

Preparation of a gel containing PMMA fragments of 500 nm to which hydrophobic antigen has been added.

25 Haemagglutinin antigen prepared from A2 virus according to Example 11 was absorbed by hydrophobic interaction on 500 nm PMMA particles. Said particles were added to 1% solution according to Example 15 and a reduction to half the volume was accomplished. A stable homogenous viscoelastic gel was formed which was useful as
30 a vaccine having a high adjuvant effect.

Example 17

A comparison between the degree of reswelling at free availability of water between conventionally prepared gels and gels prepared according to the present invention.

5 Hyaluronic acid gels prepared according to Laurent et al 1963 and according to Examples 1 and 2 above were dried to half their swelling volumes. Then they were re-introduced into their original solutions. The previously
10 known gels swelled to their original volume while the gel compositions according to the present Examples 1 and 2 swelled marginally only (10%).

Example 18

Comparison between biological activity of EPO copolymerized with hyaluronic acid to a gel and the gel according to Example 1 into which EPO had been enclosed by a concentration of said gel.

15 Four patients under treatment with Eprex® (CILAG) for their anaemia caused by chronic uraemia were treated for two months with a dose each month according to the following regimen:

Patient no.	1	2	3	4
Dose IU	60 000	70 000	70 000	50 000
Month 1	Directly gelled depot,	Directly gelled depot,	Control	Control
Month 2	Control	Control	Concen- trated	Concen- trated

Directly gelled depot: Epoxide cross-linking under mild conditions according to Example 11 in the presence of EPO.

25 Control: EPO dissolved in 4% hyaluronic acid MW about 6×10^6 from cock's comb prepared according to US 4 141 973 (Healon® Pharmacia).

Concentrated: EPO enclosed within activated gel which was gelled through concentration.

30 The dose was selected as the total dose per month which was normally required by the patient to maintain

the haemoglobin level. The serum level of EPO was analysed at regular intervals by means of an immunochemical method.

Results

- 5 A common method of expressing the functionary effect of depot preparations is to calculate the curve area (units of EPO x days). This study also gives the bioavailability in the form of haemoglobin level in blood as 0 = retained, + = increased and - = reduced.

10 Table

Patient no./month	1/1	1/2	2/1	2/2	3/1	3/2	4/1	4/2
Area under the curve	41	424	57	534	224	952	567	656
Haemoglobin control	-	+	-	+	0	+	+	+

Conclusion

- 15 An enclosure of EPO in a contracted depot gives the highest possible release during the analysis. Attempts to perform the gelling reaction in the presence of EPO destroyed the hormones such that a very low release could be registered.

CLAIMS

1. A process for preparing a cross-linked biocompatible polysaccharide gel composition, which comprises:

5 forming an aqueous solution of a water soluble, cross-linkable polysaccharide;

initiating a cross-linking of said polysaccharide in the presence of a polyfunctional cross-linking agent there-
10 for;

sterically hindering the cross-linking reaction from being terminated before gelation occurs, an activated polysaccharide thereby being obtained; and

15 reintroducing sterically unhindered conditions for said activated polysaccharide so as to terminate the cross-linking thereof up to a viscoelastic gel.

2. A process according to claim 1, wherein the
20 polysaccharide is selected from the group consisting of glucose amine glucans.

3. A process according to claim 2, wherein said glucose amine glucan comprises hyaluronic acid.

4. A process according to any one of the preceding
25 claims, wherein the cross-linking agent is selected from the group consisting of aldehydes, epoxides, polyaziridyl compounds, glycidyl ethers and divinyl sulphones.

5. A process according to claim 4, wherein said glycidyl ether comprises 1,4-butanediol diglycidylether.

30 6. A process according to any one of the preceding claims, wherein said sterically hindering of the cross-linking reaction comprises diluting the aqueous medium in which the cross-linking reaction is performed, to accomplish a lower concentration of the polysaccharide in said
35 medium.

7. A process according to any one of the preceding claims, wherein said reintroduction of sterically un-

hindered conditions comprises evaporating the aqueous medium in which the cross-linking reaction is performed, to accomplish a higher concentration of the polysaccharide in said medium.

5 8. A process according to any one of claims 1-6, wherein said reintroduction of sterically unhindered conditions comprises dialysing the aqueous medium in which the cross-linking reaction is performed.

10 9. A process according to any one of the preceding claims, wherein the initial cross-linking reaction in the presence of a polyfunctional cross-linking agent is performed at an alkaline pH, preferably above pH 9, ether cross-linking reactions thereby being promoted.

15 10. A process according to any one of claims 1-8, wherein the initial cross-linking reaction in the presence of a polyfunctional cross-linking agent is performed at an acidic pH, preferably at pH 2-6, ester cross-linking reactions thereby being promoted.

20 11. A process according to any one of the preceding claims, wherein said sterical hindrance of the cross-linking reaction is accomplished before said cross-linking agent has been consumed.

25 12. A process according to any one of the preceding claims, wherein a biologically active substance is enclosed within the cross-linked polysaccharide gel composition during the preparation thereof, preferably at physiological pH and salt concentration conditions.

30 13. A process according to claim 12, wherein said active substance is enclosed within the gel composition by dissolving or dispersing the same in said activated polysaccharide before subjecting last-mentioned polysaccharide to sterically unhindered conditions.

35 14. A process according to any one of claims 12 and 13, wherein said biologically active substance is selected from the group consisting of hormones, cytokines, vaccines, cells and tissue augmenting substances.

15. A process according to claim 14, wherein said tissue augmenting substance comprises a polymer selected from collagen, starch, dextranomer, polylactide and copolymers thereof, and poly- β -hydroxybutyrate and copolymers thereof.

16. A process according to claim 14, wherein said hormone is selected from the group consisting of erythropoietin and calcitonin.

17. A process according to any one of claims 12-16, wherein said biologically active substance contains functional groups reactive with the polysaccharide and is enclosed within the gel structure by chemical reaction therewith.

18. A process according to claim 17, wherein said biologically active substance containing functional groups has been prereacted with a cross-linking agent for said polysaccharide, preferably the same cross-linking agent as is used in the cross-linking of the polysaccharide.

19. A cross-linked biocompatible polysaccharide gel composition whenever prepared by a process as claimed in any one of claims 1-18.

20. A partially cross-linked biocompatible activated polysaccharide gel composition as obtained by a process as claimed in any one of claims 1-18 before continuing the cross-linking of the activated polysaccharide by reintroducing said sterically unhindered conditions for the cross-linking reaction.

21. A cross-linked biocompatible polysaccharide gel composition, which is obtainable by cross-linking of a cross-linkable polysaccharide with a polyfunctional cross-linking agent therefor in two steps, the first cross-linking step being terminated before gelation occurs by a sterical hindrance of the cross-linking reaction, and the second cross-linking step being initiated by reintroducing sterically unhindered conditions for

said cross-linking reaction to continue the same up to a viscoelastic gel.

22. A cross-linked biocompatible polysaccharide gel composition according to claim 21, which is defined by
5 any of the features claimed in any one of claims 2-11.

23. A cross-linked biocompatible polysaccharide according to any one of claims 21 and 22, which comprises a biologically active substance enclosed therein.

24. A cross-linked biocompatible polysaccharide according to claim 23, wherein said biologically active
10 substance is as defined in any one of claims 12-18.

25. A medical or prophylactic polysaccharide gel composition as defined in any one of claims 21-24.

26. A composition according to claim 25, which is
15 adapted as a depot preparation.

27. A composition according to any one of claims 21-24 for use as a medical or prophylactic composition.

28. Use of a composition according to any one of claims 21-24 for the manufacture of a medical or prophylactic composition for tissue augmentation of a mammal,
20 especially a human being.

29. Use of a composition according to any one of claims 21-24 for the manufacture of a medical or prophylactic depot composition, especially for hormone treatment of a mammal, especially a human being.
25

30. A method of medical or prophylactic treatment of a mammal, especially a human being, which comprises administering a composition as defined in any one of claims 25-26 to a mammal in need of such a treatment.
30

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 96/00684

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C08B 37/08, C08L 5/08, A61K 31/725

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C08B, C08L, A61K, A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPODOC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0203049 A1 (LKB-PRODUKTER AB), 26 November 1986 (26.11.86), abstract, page 5, line 5 - line 13 --	1
A	US 5128326 A (ENDRE A. BALAZS ET AL), 7 July 1992 (07.07.92), column 2, line 35 - line 49, column 3, line 47 - line 52, column 6, line 22 - line 31 --	1-4,9,12,15, 19,28
A	US 4863907 A (KATUKIYO SAKURAI ET AL), 5 Sept 1989 (05.09.89), column 2, line 31 - line 35, column 3, line 50 - line 66, abstract, claims -- -----	1-29



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

7 October 1996

Date of mailing of the international search report

24 -10- 1996

Name and mailing address of the ISA/
Swedish Patent Office
Box 5055, S-102 42 STOCKHOLM
Facsimile No. +46 8 666 02 86

Authorized officer

Agneta Österman Wallin
Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 96/00684

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 30
because they relate to subject matter not required to be searched by this Authority, namely:
Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods (see PCT Rule 39(iv))
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

INTERNATIONAL SEARCH REPORT
Information on patent family members

05/09/96

International application No.
PCT/SE 96/00684

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A1- 0203049	26/11/86	JP-A- 62025102	03/02/87
		SE-B,C- 458525	10/04/89
		SE-A- 8502574	24/11/86
		US-A- 4973683	27/11/90
US-A- 5128326	07/07/92	AU-B- 569157	21/01/88
		AU-B- 572419	05/05/88
		AU-A- 4304585	12/06/86
		AU-A- 7217387	27/08/87
		CA-A- 1230186	08/12/87
		DE-A,C- 3520008	19/06/86
		DE-A,C- 3546811	31/10/90
		FR-A,B- 2574414	13/06/86
		GB-A,B- 2168067	11/06/86
		GB-A,B- 2181147	15/04/87
		GB-A,B- 2181148	15/04/87
		GB-A,B- 2205848	21/12/88
		JP-C- 1745462	25/03/93
		JP-A- 2138346	28/05/90
		JP-B- 4030961	25/05/92
		JP-B- 6037575	18/05/94
		JP-A- 61138601	26/06/86
		SE-B,C- 460792	20/11/89
		SE-C- 501828	22/05/95
		SE-A- 8503486	07/06/86
		SE-A- 8901672	10/05/89
		US-A- 4582865	15/04/86
		US-A- 4605691	12/08/86
		US-A- 4636524	13/01/87
		AU-B- 595524	05/04/90
		AU-A- 6090386	04/06/87
		DE-A- 3684887	21/05/92
		EP-A,B- 0224987	10/06/87
		SE-T3- 0224987	
		JP-B- 6092320	16/11/94
		JP-A- 62129226	11/06/87

INTERNATIONAL SEARCH REPORT

Information on patent family members

05/09/96

International application No.

PCT/SE 96/00684

Patent document cited in search report		Publication date	Parent family member(s)		Publication date
US-A- 4863907	05/09/89	DE-A-	3583963	10/10/91	
		EP-A,B-	0161887	21/11/85	
		SE-T3-	0161887		
		EP-A,B-	0167363	08/01/86	
		SE-T3-	0167363		
		JP-A-	61164558	25/07/86	
		US-A-	4716224	29/12/87	
		JP-B-	6034814	11/05/94	
		JP-A-	61168362	30/07/86	
		JP-B-	6011694	16/02/94	
		JP-A-	61172808	04/08/86	
		JP-B-	6049723	29/06/94	
		JP-A-	61012701	21/01/86	

This Page Blank (uspto)